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Bioassay of Pituitary Prolactin in Pregnancy, Pseudopregnancy and Lactation: Hamster.

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BIOASSAY OF PITUITARY PROLACTIN IN PREGNANCY, PSEUDOPREGNANCY AND LACTATION: HAMSTER

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ABSTRACT

The pituitary prolactin content of hamsters in various physiological states (estrus females, 5, 10 and 15 day pregnant females; 2, 4, 6 and 10 day lactating females; and 2, 4, and 6 day pseudopregnant females) has been bioassayed, using the Grosvenor and Turner (1958) modification of the local tissue response method. The estrual female contains 0.0135 I.U. of prolactin per milligram of pituitary tissue. During pregnancy the prolactin levels rise to 0.026 I.U./mg. of pituitary tissue by day five, and to 0.045 I.U./mg. of tissue by day ten. The fifteen day pregnant females contain 0.041 I.U./mg. pituitary tissue. In lactating females, a maximum is reached by day two post partum when prolactin content reaches 0.047 I.U./mg. of pituitary tissue. This level also obtains on day four of lactation. The level falls to 0.035 I.U./mg. of pituitary tissue on day six and to 0.018 I.U./mg. of pituitary tissue in ten day post partum lactating females. During pseudopregnancy the pituitary prolactin content rises as in pregnancy to day two. Thereafter there is no further rise during pseudopregnancy. It is down slightly on day six. Data obtained from the bioassay of hamster pituitaries have been compared with those of other rodents. It may be concluded that except for the relatively high levels of prolactin/mg. pituitary tissue encountered during the second trimester of pregnancy, the prolactin curve for the hamster pituitary resembles that for the

rat and the mouse as recorded. The relatively high prolactin level during pregnancy in the hamster may be attributed partly to species differences and partly to the different assay methods used.

INTRODUCTION

Prolactin is one of the six well established hormones of the anterior pituitary. It is probably secreted by the acidophil cells of the anterior pituitary (Azimove and Altman, 1938; Smelser, 1941, Everet and Baker, 1945; Desclin, 1945 and Herlant, 1952). Whether prolactin is secreted by a separate class of acidophils is not definitely known, but evidence indicates that such is the case. In the cat (Dawson, 1946; Herlant and Racadot, 1957), rabbit (Pearse, 1951), monkey (Dawson, 1948) and bat (Herland, 1956a) it has been shown that carminophils exhibit prolactin-containing granules and the secretory activity of these granules varies with the condition of pregnancy and lactation. Recent histochemical studies of Barnnett, Roth and Salzer (1961), and the electron microscope studies of Hymer, McShan and Christiansen (1961) on rat pituitaries substantiate the earlier findings.

Prolactin has been reported to be present in the pituitaries of many mammals and birds, as well as in blood, urine, liver, placental tissue, cystic human breasts and cow manure (Meites and Turner, 1948a) and it is agreed that the hormone is a protein (McShan and Turner, 1937); White, et al., 1937; Evans, 1937). Prolactin with activity as high as 30 I.U. - 40 I.U. per milligram has been prepared in various laboratories (Li, et al., 1939; White, et al., 1942 and Bergman and

Turner, 1942).

Since the discovery of a lactogenic factor in the pituitary by Stricker and Grueter (1928, '29) and subsequent confirmation by Corner (1930), Nelson and Pfiffner (1930, '31), Turner and Gardner (1931), and Asdell (1931), the physiology of prolactin has been extensively studied. The prolactin-mammary gland relationship has been thoroughly investigated by Folley (1940, '52a, '52b, '54, '55, '56), Folley and Cowie (1944), Folley and French (1949, '50), Folley and Greenbaum (1947, '48), Folley, Guthkelch and Zuckerman (1939), Folley and Kon (1938), Folley and McNaught (1958, '60), Folley and Malpress (1948a,b), Folley and Young (1941), Meites (1954, '57a, '57b, '59, '60), Meites and Sgouris (1953, '54), Meites and Turner (1942a, b, c, d, e, '47, '48), Turner (1952), Turner and Gomez (1933, '34), Turner and Schlutze (1931), Turner and Slaughter (1930), and Reece and Turner (1937), among others. It has been shown on the basis of the above and many other studies that prolactin is both lactogenic and mammogenic.

In addition to the lactogenic and mammogenic activities in mammals, a luteotropic role of prolactin has been conclusively demonstrated in rats (Astwood, 1941; Cutuly, 1941; Evans, Simpson and Turpeinen, 1941; Lyons, 1942; Tobin, 1942; Fluhman and Laqueur, 1943; Everett, 1944; Sydnor, 1945; Greep and Chester-Jones, 1950a). A possible luteotropic role in women has been indicated by the studies of Bradburry, Brown and Gray (1950); and in sheep, evidence of such a role has been provided by Moore and Nalbandov (1955).

The identity of prolactin with the pituitary crop-gland stimulating factor in birds was established by Riddle and his co-workers

(1931, '32, '33). This role of prolactin has since been used for a number of useful and sensitive prolactin bioassay methods employing pigeons and doves as the test animals (see review of literature in this dissertation). Folley (1938, '39) questioned the identity of pigeon crop-gland stimulating factor and lactogenic hormone. Bergman, Meites and Turner (1942) concluded as a result of many studies that there appears to be no evidence that the factor which initiates lactation in mammals is not identical with pigeon crop-gland stimulating factor.

Pituitary prolactin levels have been accepted as an index of physiological activity (Meites and Turner, 1948). As pointed out by Reece and Turner (1937) and Meites and Turner (1948, '50), a sharp post partum rise in prolactin is exhibited by all the mammals studied so far. The post partum rise is correlated with the initiation and maintenance of lactation, a function which appears to be regulated chiefly by the amount of prolactin released into the blood from the pituitary.

The pituitary prolactin content has been determined for several rodents and other animals (Table I), but nothing is known of pituitary prolactin content of hamsters in any physiological state. The present studies have, therefore, been undertaken to determine the prolactin content of the pituitaries of normal, pregnant, pseudopregnant and lactating hamsters. It is hoped that these studies will serve to advance our knowledge of the physiology of reproduction. Especially is it important to ascertain ultimately whether or not prolactin has a luteotropic role in these hamsters.

TABLE I

MAMMALS IN WHICH PITUITARY PROLACTIN HAS BEEN ASSAYED

Animal	Normal Female	Pregnant Female	Lactating Female	Investigator and Date
1. Beef and Dairy Cattle	+			Reece and Turner, 1937
2. Cat	+			Reece and Turner, 1937
3. Goat	+			Koger, Meites and Turner, 1948
4. Guinea pig	+	+	+	Holst and Turner, 1948
5. Horse	+			Chance, et al., 1939
6. Man	+			Chance, et al., 1939
7. Mouse	+	+	+	Hurst and Turner, 1942
8. Pig	+			Chance, et al., 1939
9. Rabbit	+	+	+	Holst and Turner, 1939
10. Rat	+	+	+	Reece and Turner, 1937
11. Sheep	+			Chance, et al., 1939

(+) Assayed

REVIEW OF LITERATURE

It was demonstrated by Stricker and Grueter (1928, '29) that one principle or another principle contained in the anterior lobe of the pituitary was responsible for the initiation of milk secretion in well developed mammary glands. Grueter (1928) working in the laboratory of Ancel and Bouin at the University of Strassbourg was attempting to confirm the gonadotrophic activity of anterior pituitary extracts. He noted that in pseudopregnant rabbits those extracts stimulated not only the ovaries and secondary sex characters, but also initiated copious milk secretion in the developed mammary glands. Stricker and Grueter (op. cit.) extended these findings to dogs, cows, and hogs and noted that lactation could be induced in these animals if mammary glands were adequately developed.

This discovery was soon confirmed in America. Corner (1930) reported that the administration of extracts of whole sheep pituitary to spayed virgin rabbits caused proliferation of the mammary gland and simultaneous lactation, producing in two weeks a condition scarcely distinguishable from that near the end of gestation.

Nelson and Pfiffner (1930) confirmed the work of Corner (1930) using spayed virgin guinea pigs. A copious flow of milk was produced in three animals using only anterior pituitary extracts. The same authors (1931) again reported that the profound mammary growth of

pregnancy terminating in lactation in rabbits, rats and guinea pigs was apparently attributable to anterior lobe hormone or a substance physiologically similar.

Turner and Gardner (1931) prepared an alkaline extract of the anterior pituitary which induced lactation in mature castrated rabbits whose mammary glands had been developed previously. Even in extremely involuted glands lactation resulted following the administration of the extract.

Asdell (1931) succeeded in bringing spayed virgin rabbits to full mammary development using the alkaline anterior lobe extracts and expressed the opinion that such an extract contained a mammary stimulating substance which was qualitatively and quantitatively different from some of the other anterior lobe hormones.

Riddle and Braucher (1931) discovered that in pigeons and doves the two dorsolateral areas of the crop which normally undergo an extreme enlargement at the end of the brooding period and then secrete the "crop milk" can be induced to a complete glandular enlargement by suitable extracts of the anterior pituitary and by such extracts only. They were, however, not sure whether the principle activating the crop-gland was the growth hormone, the sex maturity hormone or a third and then unknown pituitary hormone.

Riddle, Bates and Dykshorn (1933) were the first to provide conclusive evidence that a) the effective stimulus to milk secretion in the prepared mammary gland was neither the growth stimulating hormone, nor the gonad stimulating hormone, but that the stimulus was provided by an unidentified hormone for which the name "prolactin"

was proposed; and b) that the effective stimulus for a specific enlargement and functioning ("crop milk" formation) of the crop-gland in doves and pigeons was neither the growth nor the gonad stimulating (and thyroid stimulating) pituitary principle, and that it was not obtainable in detectable quantities from tissues and fluids other than the anterior pituitary hormone which excited lactation in mammals. This discovery led to the development of a number of sensitive and useful bioassay methods using pigeons as the test animals.

One of the first methods introduced by Riddle, Bates and Dykshorn (1931) was based upon crop-gland weight. It was recommended that either the small ring doves or much larger common pigeons of any race could be used and the difficulties offered by these size differences were much simplified by the fact that crop-gland weight was shown to be directly proportional to the body weight of the bird, irrespective of race.

Eighteen doves, 2.8 to 3.3 months old were injected once daily on four successive days with equal doses of prolactin and killed on the fifth day. Both dosage and crop-gland weight were corrected for body weight (correction = $\frac{150}{\text{body weight}}$). A linear relationship was shown to exist between log doze x $\frac{150}{\text{b.w.}}$ plotted against the crop-gland weight x $\frac{150}{\text{b.w.}}$. The equation for the line so obtained was determined by the method of least squares and was found to be:

$$\text{CROP-GLAND WEIGHT} \times \frac{150}{\text{b.w.}} = 375 + 795 \left(\text{Log}_{10} \text{ Prolactin in Mg.}\right) \times \frac{150}{\text{b.w.}} .$$

The injections were given intramuscularly (in the pectoral muscle). The stimulated area of the crop-gland was excised, cleaned of adhering fat and weighed. Care was taken that nothing but the glandular area was weighed. The unit was defined temporarily as that amount of the hormone functionally equivalent to one milligram of their preparation No. 51, that being also the threshold dose per 150 gm. body weight of immature doves and pigeons. Crop-glands weighing 1150 mg. represented 10 units of prolactin.

It was soon realized (McShan and Turner, 1936, '37) that increase in crop-gland weight of common pigeons, though a valuable qualitative test, was unsatisfactory in quantitative assay of prolactin due to variability of groups of birds to the same amount of hormone insofar as crop-gland weight is concerned. It was therefore proposed (Lyons and Page, 1935; McShan and Turner, 1936, '37) that minimum proliferation instead of weight of the crop-gland was a more satisfactory criterion. The crop-gland growth is characterized by the presence of transverse strands or lobules of epithelial tissue and considerable opaqueness of the crop-gland when the crop-sac was extended and viewed against transmitted light. Taking advantage of this fact McShan and Turner (1936) proposed the "minimum stimulation" method for bioassaying prolactin. The excised distended crop-sac was examined by transmitted light. Positive stimulation was indicated by the presence of typical parallel strands of thickened mucosa. Injections were made for four days in the muscle of the pectoral region just beneath the skin. Birds were sacrificed 96 hours after the first injection. A unit was defined as "the total amount of hormone injected

during a period of four days which will cause a minimum but definite proliferation of the crop-glands of 50 ± 11% of twenty common pigeons weighing 300 ± 40 grams." The authors claimed that this was more accurate than the weight method.

Bates and Riddle (1936) noted that a subcutaneous injection would further increase the sensitivity thus lowering the minimum stimulating dose when compared with an intramuscular injection. Li and Evans (1948) employed this method routinely to estimate lactogenic potency and found that satisfactory results were present in two out of three birds. The quantity of hormone was considered one unit.

Lyons (1937) corroborated the claim that it is unnecessary to depend upon crop-gland weight increase. He employed two types of tests, the "macrotest" and the "microtest."[1] The "macrotest" was performed by injecting the hormone subcutaneously in the tail region and the response so obtained was found to be twice that resulting from intramuscular injection. "The macro unit" was then defined as the smallest amount of hormone which, when injected subcutaneously for four days in silver king squabs one month from hatching, caused a positive response (i.e., minimal proliferation) in the majority of five birds by the end of the fifth day. The dosage plotted against the crop-gland weight resulted in a fairly straight line even though only five birds were used. An approximate unit for other preparations could then be determined from such a curve. The unit was found to be contained in 0.12 mg.

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[1]The term macrotest is employed for assays in which the injection is made at a site other than directly over the crop-gland. The term microtest is employed for injection directly over the gland. Less hormone is required in the micro method.

of the preparation upon which the graph was based. At half of that dose (0.06 mg.) none of the three birds reacted positively.

The "microtest" (Lyons, 1937) was performed by injecting the hormone intradermally over the crop sac, care being taken (1) to form intradermal blebs or blisters in order to be assured that fluid was not passing into the crop lumen, and (2) to withdraw the needle carefully to prevent the reflux of fluid. One daily injection of 0.1 cc. was made for four days with a 27 gauge hypodermic needle. Different dose levels were used on opposite sides of the crop. The birds were killed twenty-four hours after the last injection and the sacs were inspected by viewing the stretched membrane against light. A positive reaction was indicated by the presence of typical parallel strands of thickened mucosa. The degree of proliferation of the crop-sac was rated 1, 2, 3, and 4. Such a rating was stated to be reproducible. In this method one crop-sac was injected with a known quantity of the standard solution and the other of the same bird was injected with the unknown. A comparative method such as this permits a rough quantitative estimation of prolactin from the pituitaries of small laboratory animals as well as from body fluids. The method is highly sensitive. Lyons (1937) claimed that the microtechnique enabled him to detect 1/10,000 of a micro unit. Lyons (1937) and Meites and Turner (1940) used this method for the assay of minute amounts of prolactin in human urine. Meites and Turner (1942) have also employed this for the detection of prolactin in the blood of rabbits.

Reece and Turner (1937) used the local micro method for assaying pituitary prolactin content of small laboratory animals. It has

been suggested by these workers that use of mature pigeons offers two advantages: (1) the crop-gland of mature pigeons is more sensitive than that of the squab, and (2) there are more folds on the crop-gland of mature pigeons, thus making it easier to localize the injection. The pituitaries were macerated with agate and mortar and suspended in distilled water. Injections were made in 0.1 ml. volumes with a 27 gauge needle over the same area of the crop-sac for four days. The pigeons were killed on the fifth day and a visual rating (stretching against light) of the degree of proliferation was made. It was found that response was directly related to dosage, up to a rating of four (an area of proliferation of 4 cm. in diameter). It was pointed out however that crop-gland responses greater than two were difficult to rate and that the most accurate quantitative results could be obtained by injecting enough hormone to induce a rating of 2 or less. The "bird unit" was defined as "that amount of the hormone which will cause the proliferation of an area of the crop-gland about the size of a nickel when injected intradermally over the crop-gland of a mature pigeon for four days, the bird being killed on the fifth day." The proliferation of an area 2 cm. in diameter was considered to be equivalent to two units. The prolactin content of the pituitaries was expressed either as bird units per mg. of acetone dried powder, or bird units per pituitary gland (anterior lobe). The authors maintained that this assay method based upon the intradermal mode of injection was the only known method whereby it was possible to determine the hormone content of two groups of powder in the same assay animal. Meites and Turner (1950) stated that this assay method has proved very useful for the assay of

pituitaries of small animals, but is subjective to some extent which indeed is the case.

Bergman, Meites and Turner (1940) compared three assay methods utilizing proliferation of the crop-gland of the common pigeon. The methods were those of McShan and Turner (1936), Reece and Turner (1937) and Lyons (1937). The McShan-Turner Unit was used as standard. It was shown that the Reece-Turner method required 4.5% (1/22) as much hormone and the minimum microunit only 0.56% (1/178) as much hormone to produce a unit response. Assay of standard prolactin indicated that the Lyons macrounits, the McShan-Turner Units and the Riddle-Bates Units were of the order of 0.75 to 1 to 1.5 respectively.

A second comparative study of the three pigeon methods, this one using International Standard (I.S.) prolactin (1 I.U. = 0.1 mg.), was made in the same laboratory by Meites, Bergman and Turner (1941). The three assay methods were the same as used in their earlier studies (Bergman, Meites and Turner, 1940). However, the routes of injection were: shallow intrapectoral, subcutaneous over the pectoral muscle, and intradermal over the crop=gland (microtest). These workers arbitrarily defined their unit as "The total amount of hormone injected over a period of four days which would cause a minimum but definite proliferation of the crop-glands of 50 $\pm$ 11% of 20 common pigeons weighing 300 $\pm$ 40 gm." The crop glands in all cases were examined independently by two workers using transmitted light. It was found that 0.1 mg. of I.S. prolactin was equal to one I.U. when the hormone was administered subcutaneously; that the shallow intrapectoral method required 1.25 I.U. for a unit response and that the intradermal microunit required only 1/160 of I.U. for a unit response. A comparison

of prolactin assay units yielded the following results: International Unit = 1 subcutaneous unit = 0.80 shallow intrapectoral units = 160 intradermal (microtest) units = 22.2 Reece-Turner Units (using specific strains of doves and pigeons) = 0.60 Lyons subcutaneous units (using Silver King squabs).

It was suggested that the two systematic methods (i.e., intradermal and subcutaneous) could be useful when large quantities of the hormone were available and that the intradermal microunit (i.e., over crop-sac) requiring 0.000624 mg., was the only practical method available to assay the prolactin content of blood, urine, body tissue and pituitaries of small laboratory animals.

Folley, Dyer and Coward (1940) analyzed statistically the results of assays by the pigeon crop-gland weight method and concluded that there is a positive correlation between crop-gland weights and body weights for a given dose of hormone. A sigmoid curve is obtained when either absolute crop-gland weight or crop-gland weight expressed as percentage of body weight are plotted against dose of hormone. The relationship between either of the above quantities and log-dose is approximately linear for total doses from 3 I.U. to 18 I.U.

In the procedure of assay used by Folley, Dyer and Coward, (1940) each bird received six daily injections subcutaneously in the axillary region, the daily dose being dissolved in 1 ml. of water. The birds were killed 24 hours after the last injection, the crop-glands were excised, scraped free of adherent tissue, fixed in Bouin's fluid for 24 hours, transferred to 70% alcohol and weighed after 2-3 hours. The accuracy of the method was claimed to be of the same order as that

of the biological methods of assay described in the British Pharmacopeia. Light was shown to have no influence on response, but temperature above 15° C. lowered the response. It was recommended that 15-20 pigeons be used for each group. Temperature should be kept close to 15° C. Body weight of the pigeon should be 260 - 360 gm. Hormone injections should be subcutaneous. Simultaneous comparison between the preparation under test and the standard preparation should be made and the results interpreted by reference to a curve relating dose to response and previously constructed in the laboratory making the assay. The calculations should be made on the basis of crop-gland weight.

Bergman, Meites and Turner (1942) adopted a procedure of bioassay similar to that of McShan and Turner (1936) excepting that injections were made intradermally over the crop-sac instead of intramuscularly. The unit was defined as "that amount of the hormone which, when injected intradermally over the same area of the crop-sac of twenty common pigeons (weight 300 $\pm$ 40 gm.), will elicit a minimum response in 50 $\pm$ 11% of the pigeons." The crop-gland in all cases was examined by transmitted light. This method has been claimed to be highly quantitative (Meites and Turner, 1950).

Hall (1944a) examined the pigeon crop-gland weight method in detail. The procedure of assay adopted was the same as that of Riddle, et al., (1933). It was noted that the slope of the regression line (relating dose with response) was valid only when stimulation produced crop-glands weighing more than 2200 to 2500 mg. Muscle extracts added to purified prolactin solution in certain proportions augmented the crop-sac response and in other proportions depressed it.

Prolactin administered subcutaneously produced about 50% more response than the intramuscular injections. No sex differences were found when prolactin was injected intramuscularly, but male pigeons responded slightly more than the females when it was administered subcutaneously.

Prolactin assay by comparison of the two crop-glands of the same pigeon after local injection was critically examined by Hall (1944b). White Craneau or White Kings were used as test animals. Injections were made intradermally for four days in a volume of 0.1 ml. each day with a 0.25 cc. tuberculin syringe and 0.5 inch needle. The birds were killed on the fifth day. Preparatory to making injections the feathers were removed in the crop-sac region. The injections were so made that a bleb or blister always resulted and the site of bleb was marked with a non-toxic dye for reference on succeeding days. The glands were rated by "arbitrarily determined designations" for estimation of "gross degree of stimulation" as reported earlier by Bates and Riddle (1940) who employed five such arbitrary gradations for the degree of response. However, Hall (1944b) found that it was possible to extend response range thus extending the response gradations to 18. The response interval was then termed "micro-value." The micro-value made it possible to distinguish the degree of proliferation (less than one micro-value) while comparing two glands from the same bird, following small differences in prolactin dosage. A 20% change in dosage was detected in the eight crop-sacs of four birds, but a 100% change of dosage was not uniformly detectable. The suggestion was also made (Hall, 1944b) that injections should be placed at the lateral border of the extended breast feather tract (his position 3), which was thought to be the diametric

center of the crop-gland. Injections at this site stimulate uniform and well-defined areas on the crop epithelium.

Tanabe, Syoda and Saeki (1954) noted that the arbitrary ratings of the degree of stimulation used by Bates and Riddle (1940) and Hall (1944b) are not satisfactory in the assay of prolactin. They (Tanabe, et al., 1954) devised a more objective method for estimation and hoped that it would be applicable on commercially available material. Assay procedure was essentially the same as that of Hall (1944b). Prolactin used was the Squibb powder containing 17.5 I.U. /mg. Pigeons of either sex weighing 300-400 gm. were used. Prolactin solutions to be compared were injected in 0.1 ml. volumes daily for four days intradermally over the symmetrically opposite sites of the crop-sac of the same pigeon volumes. The birds were killed on the fifth day. The whole crop-sac was removed, cut into halves and each half was stretched fully on a glass plate. The area of each of the two visibly proliferated regions was measured. A linear regression equation ($Y = 0.498X - 0.004$) was obtained between log ratio of dosages used (X) and log ratio of the two areas of stimulation (Y). The following equation was then introduced, relating the ratio of two dosages used ($\underline{x}$) and the ratios between two areas of stimulation ($\underline{y}$): $\underline{y} = 0.991\ \underline{x}^{0.498}$. This closely approximated $\underline{y} = \sqrt{\underline{x}}$. It was also shown that 1) the equation was not available in ranges lower than one-half of a Reece-Turner Unit; 2) the method was applicable in pigeons which have various sensitivities for prolactin stimulation by adopting the ratio between two responses areas in the same pigeon as an indicator to express the response; 3) a reliable estimate of the potency of the

unknown preparation could be calculated using the equation by injecting standard preparation over one crop-gland and the unknown over the other crop-gland of the same pigeon. The error was found to be less than 30%.

Grosvenor and Turner (1958) examined more thoroughly the micro method of prolactin assay with a view to increasing the uniformity and objectivity of the method. The two solutions to be tested were injected intradermally in a 0.1 ml. volume daily for four days over the symmetrically opposite sides of the crop-sacs of the fifteen pigeons used for each assay. The injection area was marked with a non-toxic dye to insure injection in the same area each day. Pigeons were killed 24 hours after the last injection and the entire crop was removed and cut in halves. Each half was then stretched fully against a light source and the diameter of the proliferated area was measured.

The degree of proliferation of the crop-gland following injections of various levels of prolactin resulted in the establishment of a regression line with a regression equation of $Y = 1.138 + 0.855 (\log_{10} 3X)$ and a standard deviation of 0.0956, between log dose per pigeon (x) and diameter (cm.) of proliferation obtained (Y) over a 31-fold dose range (0.00072 - 0.0224 mg. prolactin/bird). An index of precision (λ) of 0.11 was obtained. Responses that resulted from doses exceeding 0.0224 mg./bird or lower than 0.00072 mg./bird were difficult to measure. Standard deviation of Y (mean diametric response at dose X) was determined by the formula:

$$SY = \sqrt{\frac{\Sigma Y^2 - a\Sigma Y - b\Sigma xY}{n-2}}.$$

Results obtained from assay of preparations of unknown potency may be expressed in terms of mg. standard, or as I. U. simply by multiplying the value of X (mg.) obtained from the regression equation by the number of I. U./mg. of standard prolactin used. It has been claimed by Grosvenar and Turner that their method is convenient and accurate for expression of prolactin activity.

The method of Grosvenor and Turner cited above has been adopted in the present study for the bioassay of prolactin from the pituitaries of hamsters under various physiological conditions. It is possibly the only useful, accurate and convenient method available at present. Some of the virtues of the method are:

1) By keeping the route and volume of injections constant, the age and weight within uniform limits, and using the regression equation obtained from injection of the standard, the effects of variables such as race of the pigeon, season and light are reduced to a minimum.
2) The index of precision obtained ($\lambda = 0.11$) indicates a high degree of accuracy and reliability.
3) The measurement of the diameter of the proliferated area, which in most cases is circular, is not only easy, but is objective.
4) The known standard solution can be injected on one side and the unknown preparation can be injected on the other side of the crop-sac in the same pigeon. The prolactin activity of the unknown expressed in terms of mg. or I.U./mg. of the standard can be determined directly by use of a regression equation obtained from the regressions of (Y) on (x) for the standard.

5) The small quantities of prolactin contained in the pituitaries and body fluids of small laboratory animals can be accurately measured.

MATERIALS AND METHODS

Assay Animals: Pigeons eight to twelve weeks of age and of mixed races (Silver Kings, White Craneaus, White Kings, Mondaius, and others) and weighing 330 $\pm$ 30 gm. were used as assay animals. Pigeons were purchased from Louisiana breeders in groups of six to twenty-five. They were housed one to a cage and were fed Purina pigeon grain and water ad libitum. Most of the birds were kept in the laboratory not longer than two weeks including the days of bioassay. Effort was made to synchronize the time of bioassay with the arrival of birds.

Hamsters: Pituitaries from sixty-three hamsters were used in the present studies. All of the hamsters were obtained from the colony of golden hamsters (Mesocricetus auratus Waterhouse) maintained by the Department of Zoology, Louisiana State University, in an air conditioned animal house. The animals were fed Purina lab chow and water ad libitum. The hamsters were (1) female in estrus; (2) 5, 10, and 15 day pregnant females; (3) 2, 4, 6, and 10 day lactating females; and (4) 2, 4, and 6 day pseudopregnant females. Cyclic hamsters were identified by the vaginal smears (Kent and Smith, 1945). Pseudopregnancy was induced in estrous females by sterile mating. The day following the evening or night of sterile or fertile mating was counted

as day one of pseudopregnancy or pregnancy. (An animal mated on the night of the 1st day of the month would be five days pregnant on the 6th.) An animal giving birth at a given hour on the 1st day of a month was considered a 2-day lactating female at the same hour on the 3rd day.

Removal of Pituitaries: At appropriate time the animals were narcotized with ether and then decapitated. The skull was cleaned of skin and muscles. The calvaria was removed with the help of heavy scissors and the brain was scooped out. The diaphragma Sella was cut and the pituitary was extracted from the Sella turcica. The posterior lobe of the pituitary was separated from the anterior lobe if it had not already been removed with the brain. The anterior pituitaries were weighed to the nearest milligram and were either preserved in the freezing compartment of a refrigerator for later assay or the assay was commenced at once. Equivalent results were obtained with frozen and fresh pituitaries.

Pituitary Suspensions: A suspension was made by grinding the pituitaries with an agate and a mortar in a small but fixed quantity of pyrogen free distilled water (the amount of water used depending upon the number of pituitaries and the extent of dilution dictated by the physiological state of the animal). The suspension was transferred with a hypodermic syringe into a Serum bottle and stored in the refrigerator for injection during the bioassay period.

Preparation of Various Dilutions of Prolactin: The prolactin used was marked NIH - p - S - 3 with a primary potency of 15 I.U./mg. Each bottle contained 25 mg. of water soluble white prolactin powder.

A stock solution (stable for over one month if refrigerated) of 5 mg./ml. was prepared by dissolving 25 mg. of the powder in 5 ml. of pyrogen-free water. From this stock solution other dilutions were prepared as follows: 0.0292 mg/0.1 ml., 0.0146 mg./0.1 ml., 0.0073 mg./0.1 ml., 0.00365 mg./0.1 ml. and 0.001825 mg./0.1 ml. All the concentrations were prepared by the procedure outlined below for the preparation of 0.0292 mg./0.1 ml.

Stock solution: 5 mg./ml.

Dilution required: 0.0292/0.1 ml.

1 ml. stock solution diluted to 17.1 ml.

The resulting solution came to 0.292 mg./ml. = 0.0292 mg./0.1 ml.

At the time of injection this solution (0.0292 mg./0.1 ml.) was diluted in the ratio of 1 : 3 and 0.1 mg. of this dilution was given each bird each day, so that each bird by the end of the fourth day had received 0.0073 mg. x 4 = 0.0292 mg.

Assay Procedure: Assay procedure was essentially the same as that of Grosvenor and Turner (1958). Seven to twelve pigeons were used for each of the fourteen assays (11 in Table IV, 3 in Table VI). Feathers were plucked from the skin overlying the crop-sac six to eight hours before starting the injections. It has been shown (Hall, 1944b) that plucking the feathers in that region produced some relatively negligible proliferative response in the crop-gland. A known concentration of prolactin was injected intradermally on one side of the crop in volumes of 0.1 cc. per day for four days. An identical volume (0.1 cc.) of the unknown preparation obtained by the procedure

outlined above was similarly injected on the opposite side of the crop in the same bird. The injections were made with a 1 ml. hypodermic syringe and a 27 gauge needle inserted at the geometrical center of each crop-gland (Hall, 1944b), which site had been marked previously with a non-toxic dye for subsequent reference. The injections were such that intradermal bleb was always formed. The same procedure was adopted for each assay, but in each one bird was injected on both sides with pyrogen free water alone to serve as control.

The birds were killed on the fifth day twenty-four hours after the last injection. The skin was separated from the underlying crop-sac and the whole crop-sac was removed and bisected. The lining of each half was rinsed with running tap water and any adherent fat was removed. Each half was stretched by one person against light (table lamp fitted with a 100-watt bulb was used as a source of light) while an assistant measured in centimeters with a caliper the diameter of the proliferated area. When the stretched crop-gland is viewed against light the proliferated epithelium appears as an essentially circular opaque area of parallel epithelial strands which is easily measurable. The following suggestions are made to assure uniformity of results:

1) A constant volume of 0.1 ml. should be used for each injection.

2) Birds should be of uniform age (2-3 months old) and of uniform weight (330 ± 30 gms.).

3) All birds should be housed at the same location to insure equivalent conditions of temperature and light.

4) The sites of injections should not be varied.

5) The injections should be so made that an intradermal bleb is always formed. Preferably they are made in the follicles from which the

feathers have been plucked six hours earlier. The needle should be withdrawn carefully in order to prevent reflux of fluid.

6) The diameters of the proliferated crop-glands should be measured at two or three places and the average should be taken to represent the response level in one pigeon.

7) The diameters should be measured independently by more than one person to increase the objectivity of the results.

8) The desired concentrations of prolactin should be prepared fresh from the stock solution each time just before use.

9) Diametric responses of less than 2 cm. and more than 3.85 cm. are unreliable to rate and could be a source of error if included in the data.

RESULTS AND DISCUSSION

The Validity of the Assay

The average diametric responses (Y) of crop-glands to known quantities of N.I.H. prolactin are shown in Table II and have been plotted against the dose ($\log_{10}$ 3 X) in Figure 1. As a result of regression of (Y) on (X) a fairly straight line resulted with a regression coefficient (b) of 1.570, intercept (a) of 1.464 and the regression equation: $Y = 1.464 + 1.570 (\log_{10} 3 X)$. The use of such a statistical model is validated by the studies of Grosvenor and Turner (1958). These observers were able to obtain a regression line with a standard deviation of 0.096 between log-dose per pigeon and diameter of proliferation over a 31-fold dose range (.00072 - .0224). A high index of precision ($\lambda = 0.11$) was obtained. The details of this method are discussed elsewhere in this dissertation. The bioassays recorded in Table II and Figure I confirm the findings of Grosvenor and Turner (1958) with reference to the following:

1) A log-dose response is obtained.

2) The response is highly quantitative and reproducible.

3) The method is useful for detecting the small amounts of prolactin contained in the pituitaries of small laboratory animals.

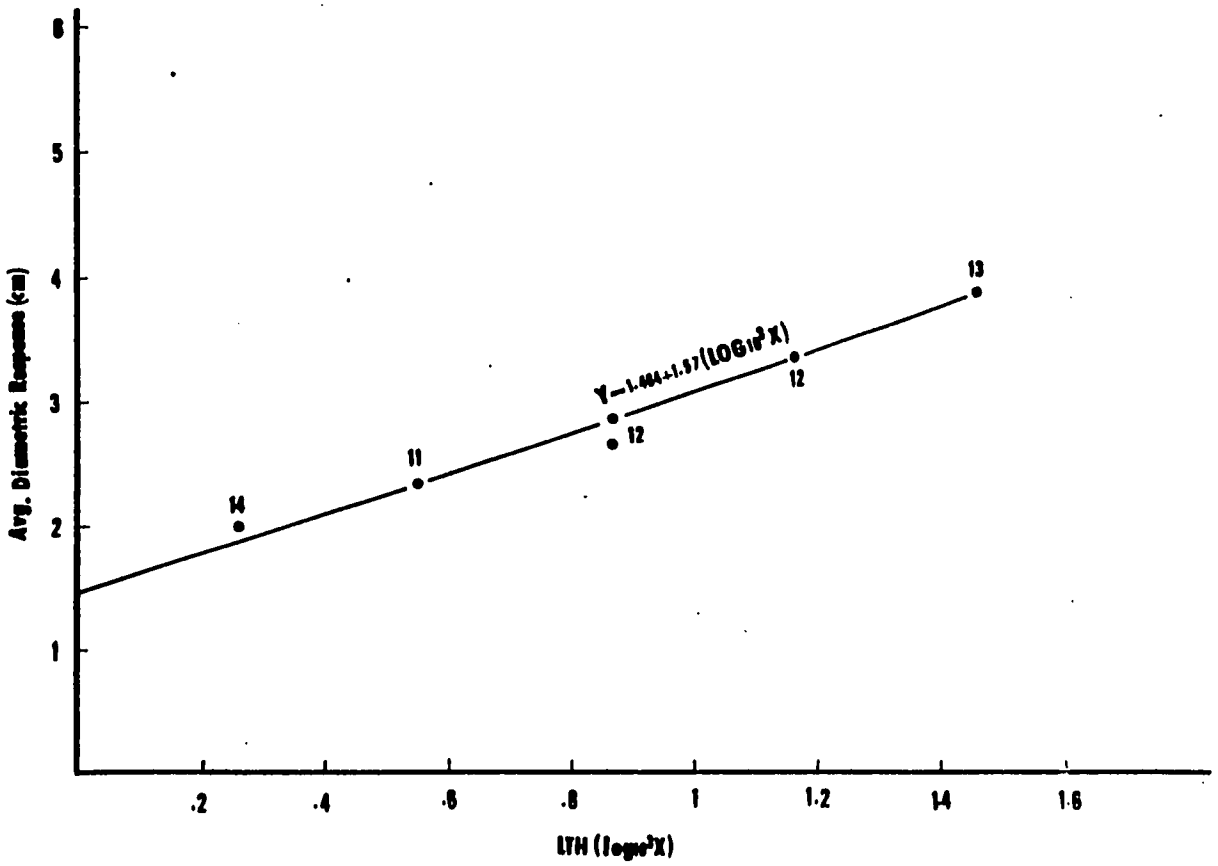

Figure 1: Average crop-gland response to known quantities of prolactin. Total dose (X) in milligrams plotted as $\log_{10} 3X$. Number of birds employed to establish each point is indicated on the points, c.f. Table II.

TABLE II

RESPONSE OF PIGEON CROP-GLAND TO FIVE DIFFERENT KNOWN CONCENTRATIONS OF PROLACTIN (P)

Test No.	No. Birds/Test And Average Weights		Total P/Bird Injected Over One Crop-gland (mg.)	No. I.U./Bird (1 mg.=15 I.U.)	Average Diameter of Response (cm.)
	n.	gm.			
1	14	338.8	0.0292	0.448	3.85
2	11	346.7	0.0146	0.224	3.38
3	12	343.5	0.0073	0.112	2.54
4	12	342.2	0.00365	0.056	2.32
5	13	340.5	0.001825	0.028	2.00

Further support of the contention that the method is highly accurate is seen in the data presented in Table III. The equation $Y = 1.464 + 1.570 (\log_{10} 3 X)$ for the regression line has been computed using sample regression equations $Y = a + bX$ and $Y = \underline{y} + \frac{S_{\underline{xy}}}{S \underline{x}^2} (X - \bar{\underline{x}})$, (Snedecor, 1959). The regression coefficient ($b = 1.570$) has been calculated from the formula $b = \frac{s_{\underline{xy}}}{s_{\underline{x}}^2}$. The sample standard deviation from regression ($s_{\underline{y}.\underline{x}} = 0.19$) has been obtained using the formula $s_{\underline{y}.\underline{x}} = \sqrt{s_{\underline{y}.\underline{x}}^2}$. A sample standard deviation of regression coefficient (s_b) is given by $s_b = s_{\underline{y}.\underline{x}} / \sqrt{\Sigma \underline{x}^2}$ and test (t) of the significance of (b) by $t = b/s_b$, d.f. = n-2 (Snedecor, 1959), where d.f. refers to degree of freedom. From the data of Table III, S_b has been found to equal 0.2 and (t) to equal 7.9,** indicating a probability < 0.01.

TABLE III

DATA EMPLOYED IN CALCULATING THE REGRESSION EQUATION IN FIGURE 1

1 Test No.	2 Dose = $\log_{10}3x$	3 Diametric Response	4 Deviation from Mean		5 Square of Deviations		6 Products of Deviations	7 Estimated $\hat{Y}$	8 Deviation from Regression	9 Square
	X	Y	$\underline{x}$	$\underline{y}$	$\underline{x}^2$	$\underline{y}^2$	$\underline{xy}$	$\hat{Y}$	$(Y - \hat{Y})$	$(Y - \hat{Y})^2 = d\underline{y}.\underline{x}^2$
1	1.465	3.85	0.602	1.032	0.362	1.065	0.621	3.764	0.086	0.0074
2	1.165	3.38	0.302	0.562	0.091	0.315	0.169	3.284	0.096	0.00921
3	0.863	2.54	0.000	-0.278	0.000	0.077	0.000	2.819	-0.279	0.0780
4	0.562	2.32	-0.301	-0.498	0.090	0.248	0.149	2.347	-0.027	0.00072
5	0.260	2.00	-0.603	-0.818	0.363	0.670	0.493	1.873	0.127	0.016
SUM	4.315	14.09	0	0	0.906 ($\Sigma\underline{x}^2$)	2.375 ($\Sigma\underline{y}^2$)	1.432 ($\Sigma\underline{xy}$)	14.087 ($\Sigma\hat{Y}$)	0	0.11133 ($\Sigma d\underline{y}.\underline{x}^2$)
MEAN	0.863($\bar{\underline{x}}$)	2.818($\bar{\underline{y}}$)								

$$\hat{Y} = \bar{\underline{y}} + \frac{s_{\underline{xy}}}{s_{\underline{x}}^2}(X - \bar{\underline{x}}) = 2.818 + \frac{1.432}{0.906}(X - 0.863)$$

$$\hat{Y} = 1.464 + 1.570\,X$$

$s_{\underline{xy}}$ + 0.19

b = 1.570

s_b = 0.2

t = 7.9** Probability $<$ 0.01

For explanation of statistical symbols see next page.

A further test of the accuracy of results was performed by calculating the correlation coefficient (r). The relationship between correlation coefficient (r) and regression coefficient (b) is stated to be: $b = r \frac{s_Y}{s_X}$ (Snedecor, 1959). (r) has been found to be 0.975 which indicates a high degree of precision. The regression equation $Y = 1.464 + 1.570 (\log_{10} 3 X)$ has been used for calculating prolactin levels in the pituitaries of hamsters in each of the physiological states studied. The results of each bioassay excepting those of pseudopregnancy are shown in Table IV. Substituting the average diametric response (Y) in the regression equation (Figure 1), the values of (X) for the corresponding values of (Y) have been obtained and expressed as I.U./mg. pituitary tissue, I.U./100 gm. body weight and I.U./pituitary. Since microunits (M.U., also known as bird units) in addition to I.U. are also in common use, equivalent M.U. values have been recorded. Appended to this paper are records of the diametric response for each pigeon used in each of the fourteen assays.

Prolactin Levels During Pregnancy and Lactation

Prolactin levels expressed as I.U./mg. pituitary tissue for virgin females in estrus, in pregnancy (days 5, 10 and 15), and in

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Explanation of Statistical Symbols Used in Table III

Mean Square deviation from regression: $s_{\underline{yx}}^2 = s_{d\underline{y}.\underline{x}}^2/n-2$

Sample standard deviation form regression: $s_{\underline{Y}.\underline{x}} = \sqrt{s_{\underline{Y}.\underline{x}}^2}$

Regression coefficient: $b = \frac{s_{\underline{yx}}}{s_{\underline{x}}^2}$

Sample standard deviation of regression coefficient: $s_b = s_{\underline{Y}.\underline{x}} / \sqrt{\Sigma \underline{x}^2}$

A test of significance of b: $t = b/s_b$, d.f. = n-2

lactation (days 2, 4, 6, and 10) are represented graphically in Figure 2. These levels have been compared with those reported from certain other species. It will be seen from Figure 2 (based on prolactin content per milligram of pituitary tissue) that there is a tendency for pituitary prolactin content in the rabbit and rat to remain fairly constant at initial low levels during the first two trimesters of pregnancy. In mice by the tenth day of pregnancy it has increased about 100 per cent above the "normal virgin female" level (Hurst and Turner, 1942) and in the hamster it has increased about 233 per cent above the estrous female level. In the "normal virgin female" guinea pig, there is a fairly high pituitary prolactin level compared to other species. The level decreases during pregnancy so that by the end of the second trimester the pituitary prolactin content is 50 per cent less than in the initial "normal virgin female." During pregnancy, the pituitary prolactin in the mouse remains constant after the initial rise, but in the hamster there is a slight fall from day ten to day fifteen of pregnancy.

A sharp preparturition?or post partum rise (Figure 2) is indicated in all species other than the hamster and, to a certain extent, in the mouse. There is an increase of only 17 per cent and 50 per cent respectively in the hamster and the mouse. In the rat, rabbit and guinea pig, there is an increase of 175 per cent, 200 per cent and 100 per cent respectively. The elevated post partum level is not maintained over a long interval in any of the species. In the hamster the pituitary prolactin content is 260 per cent above the estrous female on days two and four, but is only 33 per cent above that

of the estrous female on day ten. Prolactin levels expressed per 100 grams of body weight are reported in Figure 2.

Meites and Turner (1942, a, b, c, d, e, 1948) on the basis of rat (Reece and Turner, 1937) rabbit (Holst and Turner, 1939) and guinea pig (Holst and Turner, 1939) data considered the rapid post partum rise in pituitary prolactin of great functional significance in as much as the initiation of milk secretion is correlated therewith. With reference to the hamster and possibly the mouse the post partum rise is less impressive. Meites and Turner (op. cit.) theorized that the absence of lactation during pregnancy might be due to low levels of pituitary prolactin. They (1942 d, 1948) further concluded that the secretion of prolactin in the pituitary was kept in check by estrogen and progesterone acting synergistically during pregnancy. However, the present data reveal that in hamsters pituitary prolactin content /mg. of pituitary tissue has reached approximately 233 per cent above the estrus level by day ten of pregnancy and in the mouse the prolactin level has increased 100 per cent above that of the normal "virgin female" by day ten of pregnancy. Hurst and Turner (1942) concluded that maintenance of the non-lactating state in pregnant mice cannot be explained on the basis of pituitary prolactin levels alone. The same may be said with reference to the hamster.

Apart from the species differences it may be that the prolactin levels recorded for the rat, rabbit and guinea pig may now require revision. The validity of assay methods used for these animals has been questioned (Hall, 1944b; Folley and Malpress, 1948b). The indications were, according to Hall (1944b) that Reece and Turner were obtaining values at least 100 per cent below the absolute content in

rat pituitaries. It would not be surprising if a revised assay of the rat, rabbit and guinea pig pituitaries employing the procedure of Grosvenor and Turner (1958) and (used in the present study) were to yield higher values. As has been pointed out earlier in this paper, the Grosvenor and Turner assay method is far more objective than any other. The Reece-Turner method (1937) which was employed for assaying the prolactin content of the rat (Reece and Turner, 1939), the guinea pig (Holst and Turner, 1939) and mouse (Hurst and Turner, 1942) measured the crop-gland response in terms of subjective ratings of one to four. The method used in the present study measures the crop-gland response directly in centimeters. An explanation for the relatively high pituitary prolactin content of the hamster during pregnancy may therefore be sought a) in the species difference and b) in the difference between the bioassay methods used for the hamster and for other species of rodents.

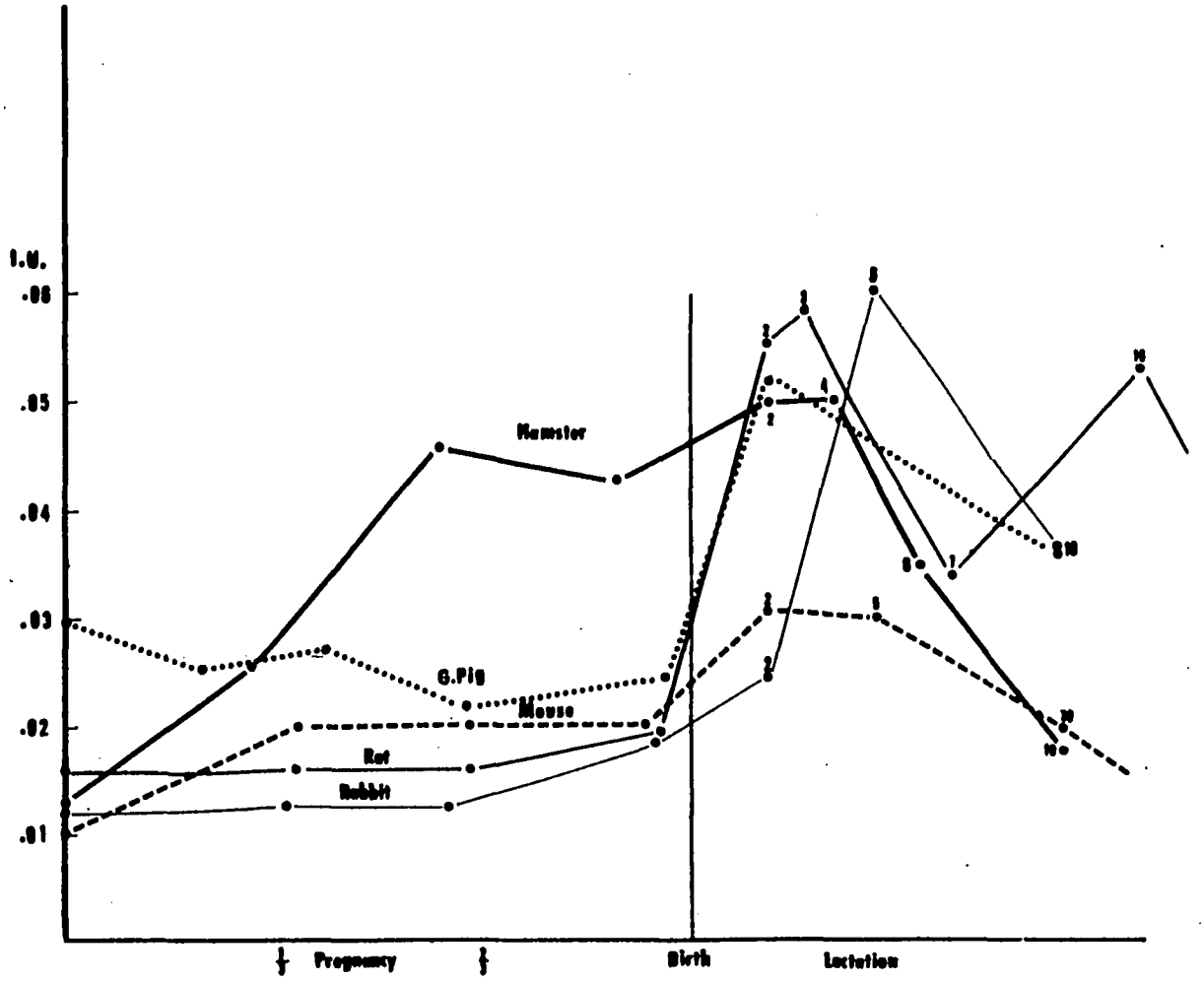

Figure 2: Pituitary prolactin content (I.U./mg. of pituitary tissue) for other rodents and the rabbit compared with hamsters in pregnancy and lactation. The initial level shown for hamsters is for virgin estrual females. Numerals on lactation end represent days post partum. (Data other than for hamster from Hurst and Turner,1942)

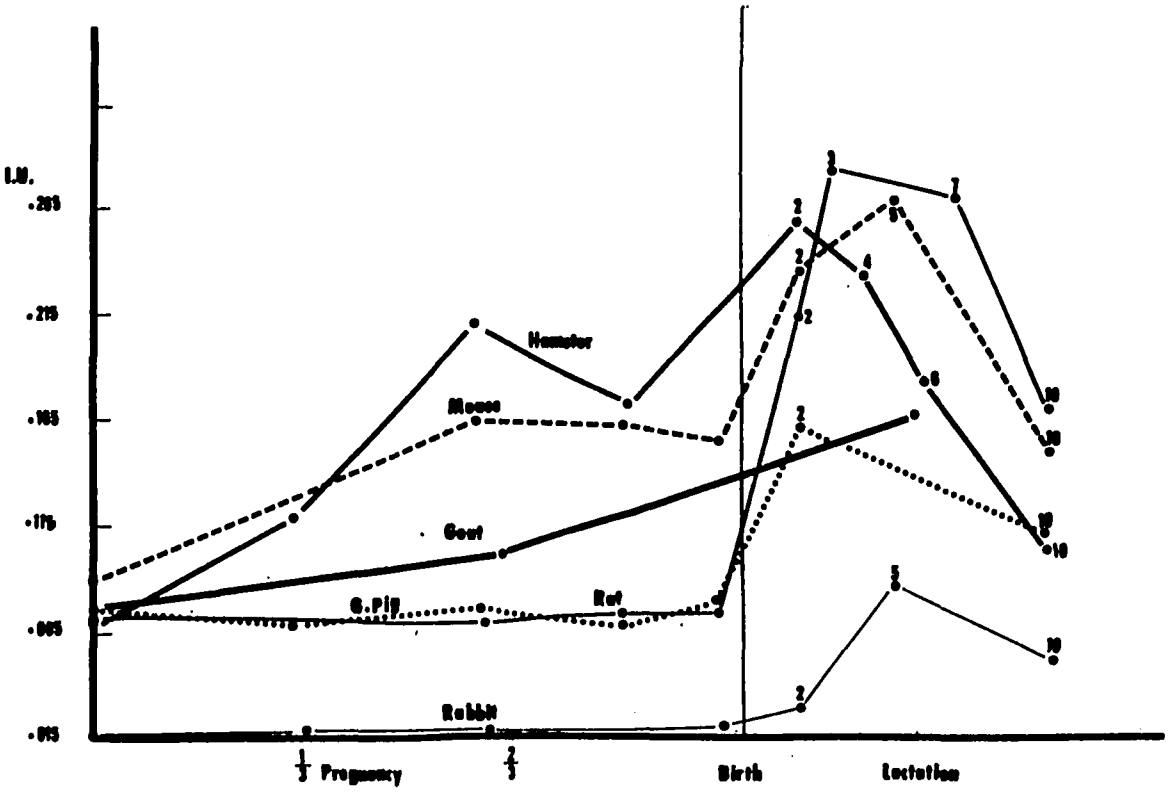

Figure 3: Pituitary prolactin content (I.U./100 gm. body weight) for various species compared with hamsters in pregnancy and lactation. The initial level shown for hamsters is for virgin estrual females. Numberals on lactation end represent days post partum. (Data other than for hamsters from Hurst and Turner, 1942).

TABLE IV

PROLACTIN CONTENT OF HAMSTER PITUITARIES IN ESTRUS, PREGNANCY AND LACTATION

International Units (I.U.): Microunits (M.U.) = 1 : 160

Assay No.	Condition	No. of Pituitary Glands	Average Body Weight (gm.)	Average Pituitary Weight (mg.)	Units/mg. Pituitary Tissue		Units/ Pituitary		Units/100 gm. Body Weight	
					I.U.	M.U.	I.U.	M.U.	I.U.	M.U.
1	Estrus (Virgin)	4	85	4.50	0.0132	2.1	0.059	9.4	0.070	11.2
2	Estrus (Virgin)	4	88	4.25	0.0140	2.2	0.059	9.4	0.067	10.8
3	5-day Pregnant	8	92	4.40	0.0260	4.1	0.114	18.2	0.124	19.8
4	10-day Pregnant	5	98	4.60	0.0460	7.3	0.211	33.7	0.215	34.4
5	10-day Pregnant	5	96	4.70	0.0450	7.2	0.211	33.7	0.215	34.4
6	15-day Pregnant	4	90	4.30	0.0410	6.4	0.176	28.1	0.180	28.8
7	2-day Post Partum	6	94	5.10	0.0477	7.6	0.243	35.0	0.257	41.0
8	4-day Post Partum	6	91	4.60	0.0473	7.5	0.216	34.5	0.237	38.0
9	6-day Post Partum	4	90	4.30	0.0380	6.0	0.169	27.0	0.187	28.9
10	6-day Post Partum	4	92	4.25	0.0330	5.3	0.150	24.0	0.162	21.9
11	10-day Post Partum	4	90	4.23	0.0180	2.8	0.076	12.1	0.085	13.4

TABLE V

INTERNATIONAL UNITS OF PROLACTIN IN THE HAMSTER PITUITARY COMPARED WITH OTHER SPECIES[1]

Species	Mature Female		Maximum During Pregnancy		Maximum Post Partum		Maximum % Increase Over Normal	
	Per mg. Pit. Tissue	Per 100 gm. Body Weight	Per mg. Pit. Tissue	Per 100 gm. Body Weight	Per mg. Pit. Tissue	Per 100 gm. Body Weight	Per mg. Pit. Tissue	Per 100 gm. Body Weight
	I.U.	I.U.	I.U.	I.U.	I.U.	I.U.	%	%
Hamster	0.0135	0.068	0.045	0.215	0.047	0.257	260	270
Mouse	0.010	0.088	0.021	0.165	0.031	0.265	210	200
Rat	0.021	0.097	0.021	0.074	0.056	0.257	175	190
Guinea Pig	0.029	0.075	0.026	0.076	0.052	0.160	90	113
Rabbit	0.015	0.015	0.019	0.019	0.059	0.081	300	400
Goat	0.060	0.075	0.092	0.103	0.124	0.157	160	109
Dairy Cattle	0.024		0.027		0.037		160	

[1]Other Species from Hurst and Turner (1942)

Pituitary Prolactin Content During Pseudopregnancy

The results of bioassay of pituitaries from 2,4 and 6 day pseudopregnant hamsters are presented in Table VI and are compared graphically in Figure 4 with the pituitary prolactin levels during pregnancy. Examination of these data shows that pituitary prolactin levels rise to about 0.023 I.U./mg. tissue on day two, an increase of approximately 70% above that of the estrual female. Compared with the prolactin level of two day pregnant females no appreciable difference is observable. In contrast, however, the pregnant female continues to register an increase in the pituitary prolactin, whereas the pituitary of the day four pseudopregnant female contains no more prolactin/mg. pituitary tissue than the day two pseudopregnant female. Between days four and six in the pseudopregnant female, there is a slight fall in the pituitary prolactin content, but it is still about 50% above the estrous level. It may be stated that during pseudopregnancy after an initial rise of about 70% above the estrual level, the pituitary prolactin content rises no further. No data except for the rabbit (Meites and Turner, 1942a) are available for pituitary prolactin levels during pseudopregnancy. Meites and Turner (1942a) found that in the rabbit pituitary prolactin content during pseudopregnancy remains low. Low pituitary prolactin content in pseudopregnancy as compared with pregnancy may provide a further clue to the many problems of reproductive physiology.

TABLE VI

PROLACTIN CONTENT OF HAMSTER PITUITARIES DURING PSEUDOPREGNANCY

International Units (I.U.): Micro Units (M.U.) = 1.160

Assay No.	Days	n.	b.w.	p.w.	Units/mg. Pituitary Tissue		Units/ Pituitary		Units/100 gm. Body Weight	
					I.U.	M.U.	I.U.	M.U.	I.U.	M.U.
12	2	4	98	4.5	0.024	3.80	0.108	17.28	0.11	17.0
13	4	3	96	5	0.022	3.52	0.110	17.6	0.11	17.6
14	6	2	100	5	0.020	3.20	0.10	16.0	0.10	16.0

n. = number of pituitaries

b.w. = body weight

p.w. = pituitary weight

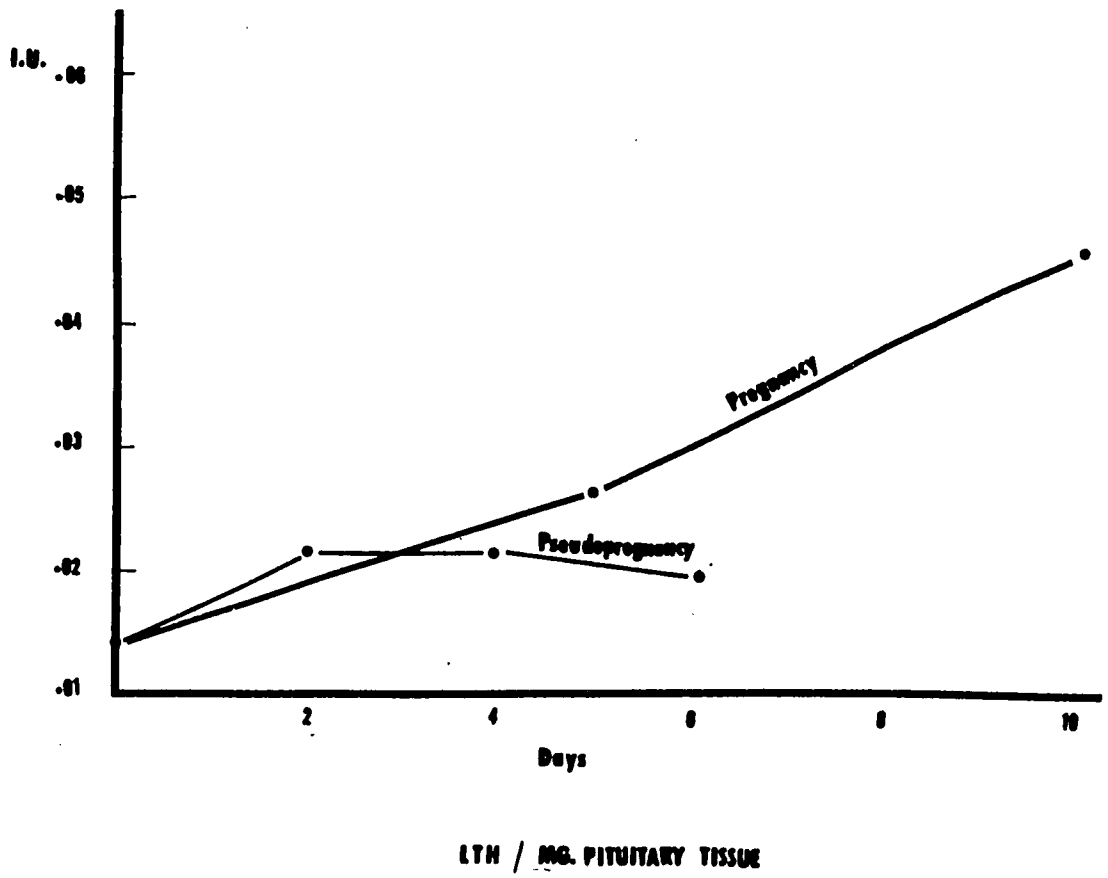

Figure 4: Pituitary prolactin content/mg. pituitary tissue during pregnancy and pseudopregnancy in the hamster.

SUMMARY AND CONCLUSIONS

1. The literature concerning pigeon assay methods has been reviewed.

2. The pituitary prolactin content of hamsters in various physiological states (estrus females; 5, 10 and 15 day pregnant females; 2, 4, 6 and 10 day lactating females; 2, 4, and 6 day pseudopregnant females) has been bioassayed.

3. It has been confirmed that the Grosvenor and Turner (1958) modification of the local method employed herein is highly quantitative and convenient.

4. The estrual female contains 0.0135 I.U. of prolactin per milligram of pituitary tissue.

5. During pregnancy, the prolactin levels rise to 0.026 I.U./mg. of pituitary tissue by day five and to 0.045 I.U./mg of tissue by day ten. The fifteen day pregnant females contain 0.041 I.U./mg. pituitary tissue.

6. In lactating females, a maximum is reached by day two post partum when prolactin content reached 0.047 I.U./mg. of pituitary tissue. This level also obtains on day four of lactation. The level falls to 0.035 I.U./mg. of pituitary tissue on day six and to 0.018 I.U./mg. of pituitary tissue in ten day post partum lactating females.

7. During pseudopregnancy the pituitary prolactin content rises as in pregnancy to day two. Thereafter there is no further rise during

pseudopregnancy. It is slightly lower on day six.

8. Data obtained from the bioassay of hamster pituitaries has been compared with those relating to other rodents.

9. It may be concluded that, except for the relatively high levels of prolactin per milligram of pituitary tissue encountered during the second trimester of pregnancy, the prolactin curve for the hamster pituitary resembles that for the rat and the mouse as recorded. The relatively high prolactin level during pregnancy in the hamster may be attributed partly to species differences and partly to the different assay methods used.

APPENDIX

The first five appended tables are records of the diametric response for each pigeon for each of the five known concentrations of the standard (NIH) prolactin injected. The averages are recorded in text Table II.

The remaining seven pages are records of the diametric responses for each pigeon used in each of the fourteen bioassays. The averages are recorded in text Tables IV and VI.

TEST No. 1

Total Prolactin Injected Over A Period of Four Days = 0.0292.

0.0073 mg./0.1 ml. each day 0.0292 mg. Prolactin = 0.448 I.U.

Pigeon No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	350	3.90
2	340	3.90
3	345	3.90
4	320	3.85
5	350	3.70
6	332	3.90
7	341	3.75
8	352	3.55
9	335	4.20
10	345	3.90
11	320	3.70
12	325	3.85
13	328	3.85
14	335	3.95
Average	338.8	3.85

TEST No. 2

Total Prolactin Injected Over a Period of Four Days = 0.0146 mg.

0.00365 mg./0.1 ml. each day 0.0146 mg. Prolactin = 0.224 I.U.

Pigeon No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	345	3.35
2	350	3.50
3	340	3.35
4	360	3.25
5	330	3.35
6	338	3.30
7	356	3.40
8	367	3.45
9	332	3.50
10	341	3.38
11	345	3.35
Average	346.7	3.38

TEST No. 3

Total Prolactin Injected Over A Period of Four Days = 0.0073 mg.

0.001825 mg./0.1 ml. each day 0.0073 mg. Prolactin = 0.112 I.U.

Pigeon No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	335	2.45
2	360	2.50
3	345	2.55
4	348	2.50
5	350	2.50
6	356	3.00
7	350	2.60
8	334	2.58
9	336	2.30
10	348	2.30
11	350	2.80
12	330	2.40
Average	343.5	2.54

TEST No. 4

Total Prolactin Injection Over A Period of Four Days = 0.00365

0.00091.25 mg./0.1 ml. each day 0.00365 mg. Prolactin = 0.056 I.U.

Pigeon No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	345.0	2.40
2	342.0	2.35
3	355.0	2.29
4	330.0	2.28
5	340.0	2.30
6	351.0	2.36
7	332.0	2.28
8	342.0	2.30
9	350.0	2.34
10	345.0	2.31
11	334.0	2.33
12	341.0	2.30
Mean:	342.2	2.32

TEST No. 5

Total Prolactin Injected Over a Period of Four Days = 0.001825 mg.

.000456.25 mg./0.1 ml. each day 0.91825 mg. Prolactin = 0.028 I.U.

Pigeon No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	340.0	2.10
2	345.0	1.90
3	335.0	2.00
4	332.0	2.00
5	342.0	2.00
6	355.0	2.05
7	355.0	2.15
8	335.0	1.86
9	340.0	2.06
10	342.0	2.00
11	330.0	2.00
12	332.0	2.00
Mean:	340.5	2.00

ASSAY No. 1			ASSAY No. 2		
Conditions: Estrus Hamsters			Conditions: Estrus Hamsters		
Dose, mg. Pituitary Tissue: 2.5			Dose, mg. Pituitary Tissue: 2.6		
Average Diametric Response: 2.00			Average Diametric Response: 2.05		
I.U./mg.Pituitary Tissue: 0.0132			I.U./mg. Pituitary Tissue: 0.0140		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	335.5	2.00	1	340.0	1.95
2	330.0	2.05	2	342.0	2.20
3	340.5	2.10	3	340.0	2.00
4	332.5	2.10	4	360.0	2.00
5	360.0	1.95	5	330.0	2.05
6	380.0	1.90	6	342.0	1.90
7	330.5	1.90	7	335.0	2.20
8	332.5	2.00			
Total	2741.5	16.00		2389.0	14.30
Av.	342.6	2.00		341.2	2.04

ASSAY No. 3			ASSAY No. 4		
Conditions: 5-day Pregnancy			Conditions: 10-day Pregnancy		
Dose, mg. Pituitary Tissue: 5.5			Dose, mg. Pituitary Tissue: 3.0		
Average Diametric Response: 3.01			Average Diametric Response: 3.1		
I.U./mg.Pituitary Tissue: 0.026			I.U./mg.Pituitary Tissue: 0.046		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	335.0	3.05	1	345.0	3.55
2	332.0	3.20	2	333.0	2.90
3	335.5	2.75	3	358.0	3.30
4	342.0	3.10	4	348.0	3.20
5	330.0	2.90	5	350.0	2.90
6	350.0	2.95	6	330.0	3.00
7	332.0	3.15	7	339.0	3.10
Total	2356.5	21.10	Total	2403.0	21.75
Av.	336.6	3.01	Av.	343.3	3.10

ASSAY No. 5			ASSAY No. 6		
Conditions: 10-day Pregnancy			Conditions: 15-day Pregnancy		
Dose, mg. Pituitary Tissue: 3.2			Dose, mg. Pituitary Tissue: 2.10		
Average Diametric Response: 3.05			Average Diametric Response: 2.63		
I.U./mg. Pituitary Tissue: .046			I.U./mg. Pituitary Tissue: 0.041		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	330.0	3.00	1	345.0	2.80
2	345.0	3.15	2	348.0	2.75
3	332.0	3.00	3	340.0	2.30
4	333.0	3.00	4	336.0	2.65
5	345.0	3.25	5	334.0	2.65
6	350.0	2.90	6	342.0	2.65
7	342.0	3.05	7	356.0	2.60
Total	2377.0	21.35	Total	2401.0	18.40
Av.	339.5	3.05	Av.	343.0	2.63

ASSAY No. 7			ASSAY No. 8		
Conditions: 2-day Post Partum			Conditions: 4-day Post Partum		
Dose, mg. Pituitary Tissue: 4			Dose, mg. Pituitary Tissue: 2.6		
Average Diametric Response: 3.20			Average Diametric Response: 2.90		
I.U./mg.Pituitary Tissue: 0.0477			I.U./mg. Pituitary Tissue: 0.0473		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	356.0	3.25	1	346.0	2.95
2	332.0	3.20	2	342.0	2.95
3	335.0	3.25	3	343.0	2.90
4	340.0	3.15	4	350.0	3.00
5	345.0	3.25	5	356.0	2.85
6	356.0	3.30	6	338.0	2.80
7	338.0	3.00	7	340.0	2.85
Total	2402.0	22.40	Total	2415.0	20.30
Av.	343.1	3.20	Av.	345.0	2.90

ASSAY No. 9			ASSAY No. 10		
Conditions: 6-day Post Partum			Conditions: 6-day Post Partum		
Dose, mg. Pituitary Tissue: 1.4			Dose, mg. Pituitary Tissue: 1.7		
Average Diametric Response: 2.40			Average Diametric Response: 2.30		
I.U./mg. Pituitary Tissue: 0.0380			I.U./mg. Pituitary Tissue: 0.033		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	341.0	2.40	1	452.0	2.2
2	352.0	2.40	2	450.0	2.4
3	340.0	2.35	3	436.0	2.3
4	344.0	2.30	4	450.0	2.4
5	338.0	2.50	5	435.0	2.35
6	330.0	2.45	6	440.0	2.20
7	341.0	2.40	7	430.0	2.25
Total	2386.0	16.80	Total	3093.0	16.10
Av.	340.8	2.40	Av.	442.0	2.30

ASSAY No. 11			ASSAY No. 12		
Conditions: 10-day Post Partum			Conditions: 2-day Pseudopregnancy		
Dose, mg. Pituitary Tissue: 2.5			Dose, mg. Pituitary Tissue: 1.8		
Average Diametric Response: 2.2			Average Diametric Response: 2.2		
I.U./mg. Pituitary Tissue: 0.018			I.U./mg. Pituitary Tissue: 0.023		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	430.0	2.30	1	325.0	2.30
2	442.0	2.20	2	360.0	2.25
3	358.0	2.40	3	330.0	2.30
4	340.0	2.10	4	350.0	2.10
5	330.0	2.00	5	418.0	2.05
6	348.0	2.20	6	350.0	2.20
7	350.0	2.20	7	330.0	2.20
Total	2598.0	15.40	Total	2465.0	15.40
Av.	342.0	2.20	Av.	352.0	2.20

ASSAY No. 13			ASSAY No. 14		
Conditions: 4-day Pseudopregnancy			Conditions: 6-day Pseudopregnancy		
Dose, mg. Pituitary Tissue: 1.7			Dose, mg. Pituitary Tissue: 1.6		
Average Diametric Response: 2.15			Average Diametric Response: 2.0		
I.U./mg. Pituitary Tissue: 0.022			I.U./mg. Pituitary Tissue: 0.020		
No.	Pigeon Weight (gm.)	Diametric Response (cm.)	No.	Pigeon Weight (gm.)	Diametric Response (cm.)
1	340.0	2.00	1	410.0	2.00
2	342.0	2.20	2	368.0	2.00
3	360.0	2.25	3	338.0	2.00
4	348.0	2.00	4	342.0	2.15
5	350.0	2.10	5	330.0	2.00
6	320.0	2.20	6	335.0	1.80
7	334.0	2.30	7	340.0	3.05
Total	2394.0	15.05	Total	2463.0	14.00
Av.	342.0	2.15	Av.	352.0	2.00

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VITA

Mahmood Husain Qazi was born in 1929 at Lahore (Pakistan). He had his elementary and secondary education at Mission High School, Lahore, and Islamia High School, Multan. He did his graduate work at Government College, Lahore, and received the B.S. degree from the University of the Panjab in 1960. He was awarded the M.S. degree in Zoology by the University of Karachi in 1956. He was a research fellow of the University of Karachi from 1956 - 58. In 1958 he joined the Zoology faculty, University of Karachi, and is still holding the same position. The United States Education Foundation in Pakistan selected him as a Fullbright Fellow in 1960 for higher studies in Zoology. He was simultaneously granted study leave by the University of Karachi. He was admitted to Louisiana State University in the fall of 1960 for graduate studies in Vertebrate Zoology and Physiology. At present he is a candidate for the degree of Doctor of Philosophy. He was married to Fahmeda in 1959.

EXAMINATION AND THESIS REPORT

Candidate: Mahmood Husain Qazi

Major Field: Vertebrate Zoology

Title of Thesis: Bioassay of Pituitary Prolactin in Pregnancy, Pseudopregnancy and Lactation: Hamster.

Approved:

George Kent Jr
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

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Date of Examination:

7/15/62